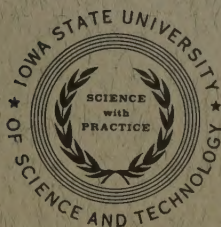


IOWA STATE JOURNAL of SCIENCE

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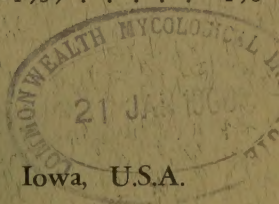


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AGE AND GROWTH OF CHANNEL CATFISH FROM THE
DES MOINES RIVER, BOONE COUNTY, IOWA, 1955 and 1956¹

R. Jess Muncy²

Department of Zoology and Entomology
Iowa State University of Science and Technology
Ames, Iowa

Abstract

Age and growth data for channel catfish, Ictalurus punctatus (Rafinesque), from the Des Moines River were determined by three independent methods. Calculated lengths at the first two annuli as determined from pectoral spine cross-sections were shorter than modes for length frequency data. Several factors were encountered in the spine technique which could cause such variations. Recorded increases in total length of some tagged channel catfish exceeded the calculated average increments. In general, the three methods substantiated each other. Less growth occurred during 1955 than 1956 probably due to decreasing summer water levels in 1955. Weight increases more rapidly than the cube of the total length. The number of eggs per mature female ranged from 2,682-9,731 and increased with age, length, and weight of the fish.

Introduction

Age and growth data were collected on channel catfish taken from the Des Moines River, Boone County, Iowa in the vicinity of the Y.M.C.A. Camp (R.27W, T.85N, Sec.35 and 36; R.27W, T.84N, Sec.1 and 12) during 1955 and 1956. Stream bottom is chiefly sand-gravel, with some rubble and boulders and with sand-silt in pools. During low-water stages in late summer and fall as much as one-half of the river bed may be exposed. The only water exceeding six feet in depth at these times is in a few scattered holes.

Ages of the catfish were determined by counting the rings on cross-sections from pectoral spines. The use of spine cross-sections for the determination of age and calculated growth of channel catfish was first described by Sneed (1951).

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²Now Fishery Biologist, Chesapeake Biological Laboratory, Solomons, Maryland.

Determination of Growth from the Spines

Pectoral spines were removed from the channel catfish in the manner described by Sneed (1951) and stored in coin envelopes. Thin cross-sections of 0.5 millimeters or less were cut from the dried spines with dental separating saw blades. Sections were obtained just above the visible terminal portion of the basal groove on the pectoral spine. Projections of the sections were enlarged for viewing by means of a "Bioscope." Transparency was increased by soaking the smaller sections in water and the larger sections in alcohol. Alcohol increased the differentiation of translucent and opaque zones (Probst and Cooper, 1955).

Sneed (1951) stated that more accurate measurements of the annuli could be determined if measurements were made along the expanded edge of the spine cross-sections. Marzolf (1955) made comparisons of age and growth measurements using pectoral spines and vertebrae. (Appelget and Smith, 1951, used rings on the vertebrae for aging catfish.) After using the expanded posterior portion of spine cross-section, Marzolf suggested that measurements along the anterior radius of the spine would give better results. The annuli on the anterior radius are often so crowded as to be difficult to distinguish, however. In the present study, relative positions of annuli with respect to center of the lumen and the maximum posterior edges were marked where the outer edge of the light zone (transmitted light) intercepted a tab board strip placed over the projected image. In some instances, the first annulus intercept could not be detected on the expanded posterior portion of the spine cross-sections of older fish. The erosion and loss of the first annulus in spines from older fish has been discussed by Marzolf (1955).

Several variables were noted in the technique of preparing and viewing the spine cross-sections which would affect the calculation of a body-spine relationship.

1. Difficulties arise in trying to obtain the section from the same location in all spines. Since the spine tapers and becomes rounded rapidly, adjacent sections decrease in size. Marzolf (1955) illustrated the continuous outward change in the basal groove with growth. Thus, spine sections cut in relation to the end of the basal groove would be located farther toward the tip with increased age.
2. The curved shape of the spine causes difficulty in obtaining perpendicular sections. Harrison (1955) discussed the problem of cutting the spine sections in the same plane and position.
3. The center of the larger lumen in the spine of older fish was more difficult to determine. As previously mentioned, the first annulus did not extend around the lumen of cross-sections from older fish. In these instances, the questions arose as to whether the center of lumen or an estimate of the center of the annulus should have been used as the base of spine-radius measurements.

4. The maximum expanded portion of all annuli did not always lie in a straight line along the maximum radius of spine.
5. The first annulus was approximately circular around the lumen; whereas the other annuli extended more into the expanded posterior portion of the spine. In age group II fish, the first annulus width would not be out of proportion as much as when compared to other annuli of older fish.
6. The projection instrument, a "Bioscope," introduced an error into the reading of annuli marks and spine radii. Magnification by the projector varied in the image field. Using medium power lens (10x) at the same focus, magnifications of 70 times were obtained in the center but magnifications ranged up to 90 times at the edge or 28 per cent more than in the center of the image field. This distortion increased progressively from the center to the edge of the field. Examination of the image field of other projection equipment has revealed that this distortion was not confined to the equipment used in this study.

All of the above variables caused greater errors in measuring the radii and annuli of larger cross-sections of older catfish. It was considered unwise to compute the body-spine relationship and make a mathematical correction since these errors are not constant for fish of all size ranges. An approximate growth rate was computed for each fish on the assumption that the growth in cross-section of the spine was directly proportional to growth in length of the fish.

Growth Rates of Channel Catfish

Calculated growth was determined from pectoral spines of 504 channel catfish collected with nets and electric shocker during 1955 and 1956 (Table 1). Three additional pectoral spine cross-sections were discarded as atypical. Two sections had regenerative growth while the third section had an excessive calculated length (19.8 to 25.1 inches) between annulus eight and nine. Because the body-spine relationship was subject to many variables, the calculated lengths in Table 1 should only be considered approximations. Probably fewer errors are present if comparisons are made of the last two or three seasons of growth (Table 2). These data indicate that growth was greater in 1954 than in 1953 and 1955.

Additional data on the growth rates in 1955 and 1956 were obtained from length-frequency data of hoopnet and wire trap catches (Figs. 1, 2). The age classes through II are fairly evident from the size distributions. The lengths of the age group I fish in the fall are somewhat greater than the computed lengths at the second annulus (Table 1), and the same is true for the age group II fish with respect to the third annulus, but the differences are not great and may easily be caused by the factors considered in the previous section. Comparisons of peaks at the same time of year in 1955 and 1956 indicate that growth (total length) was greater in 1956. Increase in total length was much slower after July in 1955, whereas growth occurred over a longer period in 1956.

Table 1. Average calculated total length (inches) at each annulus from pectoral spine cross-sections of channel catfish taken in 1955 and 1956 from Des Moines River, Iowa.

Year class	Age group	Number of fish	1	2	3	4	5	6	7	8	9	10	11	12	13
1955	I	27	2.1												
1954	II	191	1.7	5.0											
1953	II	1	2.4	5.6											
	III	79	1.9	5.4	8.0										
1952	III	25	1.9	5.3	8.9										
	IV	40	2.1	4.8	8.6	10.9									
1951	IV	17	1.9	4.5	7.3	10.6									
	V	14	1.9	4.3	7.1	10.6	12.7								
1950	V	6	2.0	4.3	7.0	9.2	12.0								
	VI	15	-	4.3	7.3	9.6	12.5	14.5							
1949	VI	12	-	4.7	7.3	10.1	12.7	15.9							
	VII	23	-	4.5	7.3	9.9	12.4	15.5	17.6						
1948	VII	12	-	4.4	7.2	10.0	12.3	14.6	16.9						
	VIII	19	-	4.1	6.6	9.1	11.8	14.5	17.4	19.2					
1947	VIII	12	-	5.0	7.2	9.8	12.4	14.9	17.2	19.1					
	IX	4	-	4.7	6.9	9.4	11.8	14.7	17.3	19.2	20.8				
1946	IX	4	-	4.6	6.3	8.5	10.9	13.6	16.3	18.9	20.9				
	X	1	-	4.6	6.0	9.3	12.4	16.3	19.4	21.4	23.8	25.2			
1945	XI	1	-	5.3	7.8	12.5	16.6	19.2	21.6	23.2	24.8	25.6	26.9		
1943	XIII	1	-	4.9	7.8	13.0	15.4	16.6	19.0	20.6	21.6	22.2	23.8	25.2	26.6
Av. calculated length			1.8	4.9	7.7	10.1	12.3	15.0	17.4	19.3	21.5	24.3	25.4	25.2	26.6
Av. annual increment			1.8	3.2	2.9	2.7	2.6	2.7	2.4	1.9	1.8	0.9	1.4	1.4	1.4
Calculated weights (lbs.) ¹⁾			0.002	0.03	0.13	0.31	0.57	1.07	1.70	2.35	3.30	4.85	5.57	5.43	6.44
Weight increments			0.002	0.03	0.10	0.19	0.30	0.50	0.63	0.65	0.79	0.54	0.91	0.89	1.01

¹⁾ Log Weight = -1.6561 + 3.1334 Log total length.

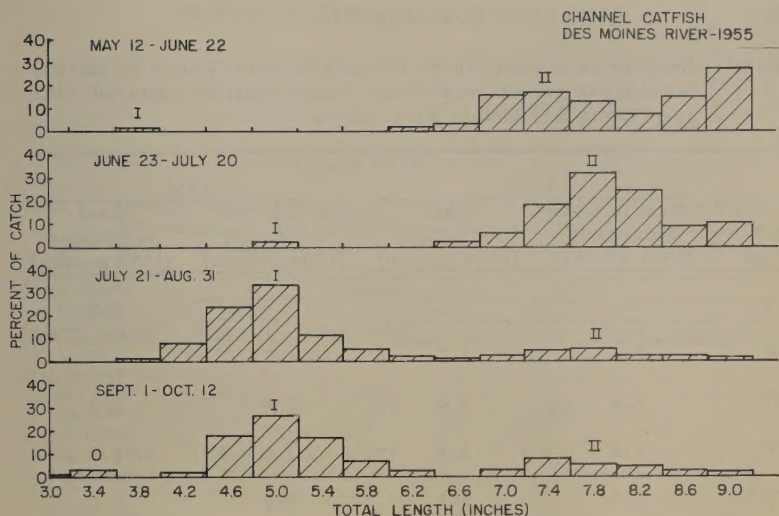


Figure 1. Length frequencies of channel catfish in hoop net and wire tra. catches for 1955, Des Moines River, Iowa.

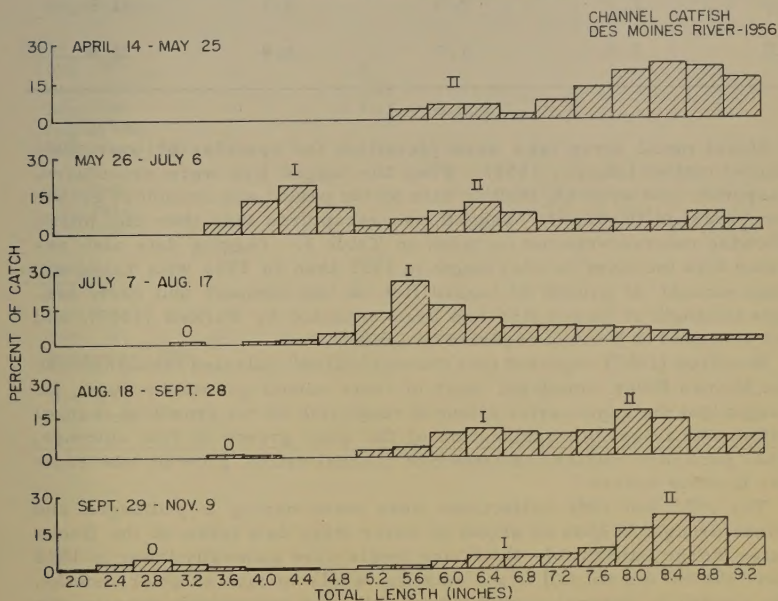


Figure 2. Length frequencies of channel catfish in hoop net and wire trap catches for 1956, Des Moines River, Iowa.

Table 2. Average calculated growth increments (total length in inches) for last two growth years from channel catfish captured 1955 and 1956 in Des Moines River, Iowa.

Age group	Year caught			
	1955		1956	
	Next to last increment (1955 growth)	Last increment (1954 growth)	Next to last increment (1954 growth)	Last increment (1955 growth)
I	-	-	-	2.1
II	2.4	3.2	1.7	3.3
III	3.4	3.6	3.5	2.6
IV	2.8	3.3	3.8	2.3
V	2.2	2.8	3.5	2.1
VI	2.6	3.2	2.9	2.0
VII	2.3	2.3	3.1	2.1
VIII	2.3	1.9	2.9	1.8

Monel metal strap tags were placed on the opercles of over 3000 channel catfish (Muncy, 1958). When the tagged fish were recaptured, measured, and weighed, further data on the period and amount of growth were secured (Table 3). Tagged fish recaptured less than one month following release were not included in Table 3. Tagging data also revealed less increase in total length in 1955 than in 1956 with relatively small amount of growth of tagged fish in late summer and early fall. Loss in length of tagged fish has been reported by Purkett (1957), and was observed in 9 cases reported in Table 3.

Harrison (1957) reported that channel catfish collected throughout the Des Moines River completed most of their annual growth by July. He thought that the suppressive effect of rough fish on the growth of channel catfish was a possible explanation of the slow growth in late summer. Other published studies indicated that channel catfish grow in late summer in other waters.

The 1955 and 1956 collections were made during a prolonged and severe drought in Iowa as shown by water stage data taken at the Boone Water Works Dam (Table 4). Water levels were generally lower in 1956 than 1955 but did not fall to the extreme levels in late summer months. The growth of channel catfish apparently was maintained later in the summer in 1956 and 1955, perhaps as a result of fairly stable water levels. Water levels in the study area are a primary factor in changing the habitat and indirectly the competition.

Table 3. Mean increase in total length in inches of channel catfish between tagging and recaptures, Des Moines River (Numbers in parentheses indicate number of recaptured fish. All others represent single recaptures.)

Total length at tagging	Recapture dates					
	1955	1956				
Date of tagging	Sept.-Oct.	April-May	June	July	August	Sept.-Nov.
<u>7-10 inches</u>						
May 1955	0.7	2.9(2)	-	-	-	3.9
June 1955	-	0.6(4)	1.2	3.5(2)	-	2.4
July 1955	0.4	0.3	-	-	-	-0.1
August 1955	-0.1(2)	0.2(7)	1.3	1.4	-	-
September 1955	0.1	0.	0.8	-	-	2.4(2)
April 1956			-	1.3	-	2.8
May 1956				1.4(2)	1.8(3)	3.3
June 1956				-	0.7(3)	1.7(4)
July 1956						0.5(8)
August 1956						0.2
<u>10-15 inches</u>						
May 1955	-	3.8	-	-	-	-
June 1955	-	1.1(3)	-	-	-	-
July 1955	-	0.9(2)	0.4(2)	-	-	-
August 1955	-	-	-	-	-	-
September 1955	-	0.1(2)	-	-	-	-
May 1956				0.9(3)	-	2.3(3)
June 1956				0.1(2)	0.3(2)	1.0(3)
July 1956						0.3(5)
<u>15-20 inches</u>						
May 1955	-	-	1.0	-	-	-
June 1955	-	-	0.1	-	-	-
July 1955	-0.3	-0.9	0.0	-	-	-
September 1955		-0.3	-	-	-	-
May 1956			-	-0.3	-	-
June 1956			0.4	-	0.2	0.3

Table 4. Comparison of Des Moines River 1955-56 average monthly water level stages with 15-year average (1933-47 inclusive)

Year	Water gauge reading in feet				
	May	June	July	August	September
15 year average	2.1	2.8	1.4	1.1	1.2
1955	1.1	1.0	0.85	0.25	0.20
1956	0.46	0.42	0.40	0.40	0.27

Length-Weight Relationship

Ponderal indexes (C) of all channel catfish for which lengths and weights were available were computed as follows:

$$C = \frac{W \times 10^5}{L^3}$$

where W = weight in pounds

and L = total length inches.

Average C values increased with the length of the fish, at least after 13 inches total length (Table 5). Comparison of ponderal indexes for fish collected in different months failed to indicate consistent seasonal trends. Tagged fish appeared to have about the same ponderal indexes at recapture as untagged fish of the same lengths.

The mathematical relationship between total length and weight can be described by the formula:

$$\text{Log } W = -1.6561 + 3.1334 \text{ Log } L.$$

For ease in computing the constants in this formula, five channel catfish were chosen at random from each half inch size group containing more than five individuals and all specimens were used in the other half inch size groups. The regression line was then fitted to the logarithms of length and weight of 180 fish. The regression coefficient (3.1334) was found to be significantly greater than 3.0 at the 0.01 level of probability ($t = 4.85$ with 178 degrees of freedom) and therefore it may be said that the weight increases more rapidly than the cube of the total length. The correlation coefficient (r) was 0.993.

Conversion factors for various length measurements of the channel catfish were determined from 421 fish arranged by half-inch size groups, ranging from 3 to 23 inches total length. Since there was no tendency for the ratios of standard to total lengths or fork to total lengths to be correlated with the length of the fish, the average ratios give the best conversion factors:

Table 5. Average weights, calculated weights, and average ponderal indexes for channel catfish, Des Moines River, Boone County, 1955-56.

Average total length (inches)	Number of specimens	Ponderal index		Weight in pounds	
		Mean	Range	Mean	Calculated ¹
3.6	4	29	20-36	.015	.012
4.3	5	29	23-35	.024	.021
5.4	15	32	24-38	.050	.044
6.6	23	30	25-38	.090	.082
7.5	37	30	14-36	.130	.122
8.5	139	29	24-45	.173	.180
9.4	92	28	18-38	.231	.247
10.4	57	29	20-38	.325	.339
11.4	36	29	20-47	.425	.452
12.5	20	27	15-35	.521	.604
13.4	30	29	16-45	.697	.751
14.4	13	32	25-39	.948	.941
15.4	9	31	26-37	1.148	1.161
16.6	13	32	27-42	1.418	1.469
17.5	8	32	18-37	1.664	1.733
18.3	18	35	24-47	2.115	1.994
19.4	8	36	30-40	2.600	2.394
20.4	12	37	32-43	3.172	2.801
21.4	7	32	27-41	3.301	3.255
22.4	4	37	34-39	4.22	3.760
23.4	4	38	34-41	4.86	4.31
24.3	1	35	-	5.06	4.35
25.1	1	45	-	7.06	5.37
26.4	2	47	47-48	8.78	6.18
Total	558	29.7	14-48	-	-

¹ $\text{Log } W = 1.6561 + 3.1334 \text{ Log } L$

Fork length = 0.876 total lengths
 Standard length = 0.734 total lengths

Fecundity

The numbers of eggs in the ovaries from 12 mature female catfish increased with the age, length, and weight of the fish (Table 6). The mathematical expression of the regression of number of eggs (Y) on total length of the fish in inches (X) is:

$$Y = -5202 + 718.4X$$

with a correlation coefficient (r) of 0.897.

The regression of number of eggs on weight of the fish in grams (W) is:

$$Y = 2.597 + 5.256W$$

with a correlation coefficient of 0.855.

Approximately 15 per cent of the total weight of mature females was eggs.

Table 6. Egg counts of channel catfish collected in the Des Moines River, Boone County, June 5 to July 16, 1956.

Age group	No.	Standard length (inches)	Total length (inches)	Weight (grams)	Weight of eggs (grams)	Number of eggs
V	2	9.0-9.2	11.8-12.3	237-258	38-45	2682-4036
VI	3	9.2-10.0	12.1-16.7	270-794	41-164	2729-7233
VII	4	11.8-13.8	15.1-18.2	482-1077	91-147	5973-7547
VIII	2	14.2-16.5	17.9-20.2	737-1389	118-235	6884-9721
IX	1	13.7	17.5	879	155	8299

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A QUANTITATIVE DETERIORATION RATING SCALE
FOR SHELLED CORN¹R. W. Lichtwardt² and G. L. Barron³

One problem in attempting to devise a quantitative evaluation of the degree of deterioration in a sample of corn lies in defining the entity which one wishes to measure. Probably no definition of deterioration of grain would find universal acceptance, for it would depend in part upon the intended use of the grain and upon establishment of certain basic standards of quality with which degrees of spoilage could be compared. Perhaps a definition of deterioration, as applied to harvested field corn which is not to be planted, might be stated as any unfavorable changes in the grain which decrease the quantity or quality of its food value or palatability, or which lessen its suitability for storage or processing.

The grading standards adopted by the grain trade are of little value to the investigator studying spoilage in grain, for the grading system depends only in part upon actual deterioration (in fact, only a few symptoms of spoilage are considered), and may lean heavily upon practical considerations. For instance, the presence of stones or cinders in a sample of otherwise good corn reduces the grain to sample grade; this foreign material may be very important in marketing, but is of little consequence to the condition of the grain per se. There is also an element of artificiality in the present standards, as exemplified in cases which have come to our attention in which stored grain, which had developed outward signs of molding (indicating probable intense internal mold activity), had been considerably upgraded, such as from No. 3 to No. 1 yellow corn, subsequent to routine transfer from one bin to another. In such cases the general appearance of the grain may have been improved due to drying and cleaning of the kernels and loss of odor and foreign material, but the possible irreversible chemical and physical damage to the kernels by the molds was not considered.

Microorganisms are known causal agents of deterioration in grain, and it is probable that in the vast majority of cases, under normal storage practices, fungi are the primary instigators of spoilage, or at least play an important secondary role in the process. Fungi probably also are directly or indirectly responsible for most of the chemical changes

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² Present address: Department of Botany, University of Kansas.

³ Present address: Department of Botany, Ontario Agricultural College, Guelph, Ontario.

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which have been used as indices of deterioration in stored grain, such as increases in fat acidity and reducing sugars or decreases in nonreducing sugars.

In the course of our studies of deterioration of shelled corn we have found it desirable to be able to assign a value to a sample of grain which indicates the extent of undesirable change or deterioration which the grain has undergone. The total presence of fungi in the kernels has not always been a satisfactory measure of deterioration, because all species or genera of molds do not seem to affect the quality of corn in an equal manner. Diplodia and Fusarium, for instance, may be present in a large percentage of kernels in a bin of grain which by all other criteria is in excellent condition. These fungi are capable of invading the kernels prior to harvest and may remain viable in dried grain for years after it is stored without any apparent damage to the grain. On the other hand, an equal percentage of kernel infection by certain species of Aspergillus and Penicillium is a rather good sign that the corn has undergone unfavorable storage conditions, and consequently some deterioration, either in the crib or in the bin, for the species in question normally do not invade kernels in the field to any great extent.

One criterion which we feel can help to determine grain quality is the amount of infection by two genera of fungi, which infection we designate as the Aspergillus-Penicillium value, or simply the A-P value. The A-P value consists of the summation of the percentages of kernels infected internally by each species of Aspergillus and Penicillium in the sample. Since individual kernels may be infected by more than one species, the summation of the percentages of infection may exceed 100. Approximately the same relative values can be obtained by using only species of fungi belonging to the Aspergillus glaucus group and the Penicillium cyclopium - P. viridicatum series, but in some instances, especially in more advanced cases of deterioration, other species of Aspergillus and Penicillium may be significant. Using all species of the two genera has a practical advantage in that a distinction need not be made between species, and the genera themselves are easily distinguished from other fungi associated with corn.

Fungi alone are not necessarily an indication of the extent to which a batch of grain has deteriorated, even when the kinds of fungi as well as their frequencies are considered. There are some instances in which the grain obviously has suffered from deterioration processes, as measured by other standards, and yet the kernels contain relatively few fungi; some of the ways in which this can happen are suggested later. In such cases, the germination percentage of the grain usually is low, and germination probably is the most direct means of measuring these unfavorable changes in the grain.

Fig. 1 is a chart which plots the percentage germination of a sample of grain against the A-P value found in the sample. The vertical and horizontal divisions of the chart were selected after consideration of all the data which we had from over six hundred samples collected in Iowa over a period of two years. These samples represented all degrees of deterioration of corn stored in all commonly-used types of bins located in scattered areas throughout the state with differing climatic and other storage conditions.

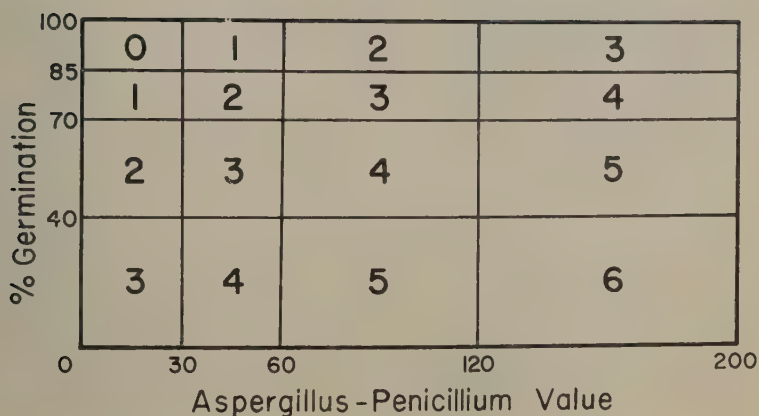


Fig. 1. Deterioration chart for shelled corn, based on percentage germination and A-P value (see text). The deterioration ratings range from 0 (excellent condition) to 6 (extremely deteriorated).

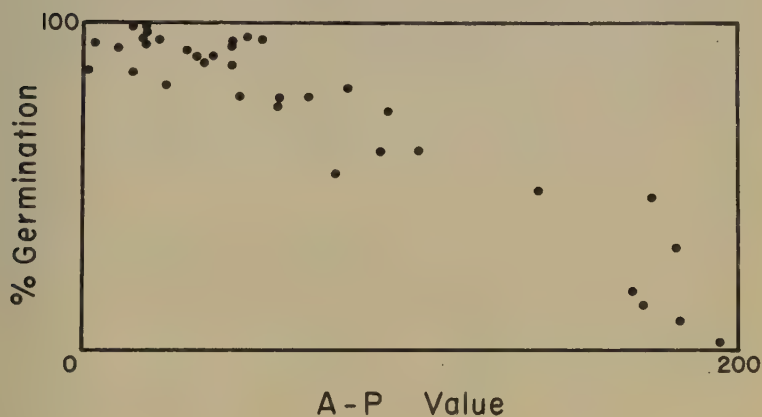


Fig. 2. Deterioration pattern of shelled corn from some farm bins (undisturbed grain) located in central Iowa.

The numbers in each box designate the deterioration rating of the samples which fall within them. The ratings range from 0 (excellent condition) to 6 (extreme deterioration). Each number might be described as follows:

0 = excellent	4 = moderately deteriorated
1 = good	5 = deteriorated
2 = fair	6 = extremely deteriorated
3 = slightly deteriorated	

Fig. 2 shows a deterioration pattern of shelled corn based on averages of all samples collected over a period of two years from eighteen bins located in central Iowa (Story County). Averages of the upper 1 foot of grain and grain from a level of 4-5 feet below the surface are shown separately for each bin. It can be seen that these averages are rather well-correlated, considering the many other factors that might influence either of the two variables used.

The original reason for devising this deterioration rating scale was for use with grain from bins of undisturbed corn, that is, corn which is not regularly stirred or otherwise excessively handled during the storage period. We found, however, that the ratings proved satisfactory when we plotted the results we had obtained from shelled corn stored in Commodity Credit Corporation binsites, where the upper grain layers normally are stirred at regular intervals and where more consideration is given to insect control. But there was a tendency in some cases for the germination of the samples to be low. At the same time there was no corresponding large A-P value as might ordinarily be expected. Instances of this sort are illustrated in Fig. 3.

Fig. 3 represents averages of data from thirteen bins located in CCC binsites in central Iowa (Story County). The averages for each bin are shown separately for the upper 1 foot of grain and grain from the 4-5 foot level. The years of harvest were from 1952 to 1955, and collections were made within a two year period. The surface grain in some of the bins had shown evidence of considerable molding and had been stirred as often as seven times during one month in order to break up the surface crust and reduce moisture.

The phenomenon of low germinability in grain, and which has at the same time a relatively low A-P value, might be explained in many ways, any of which could account for the deviations in Fig. 3 and similar cases:

1. Certain types of insecticides when applied to corn in bins may kill the kernels, especially if the grain is not particularly dry. In addition, it is possible that fungi in the kernels might also be killed by strong concentrations of insecticides.

2. Overheating of the grain during artificial drying, or otherwise improperly drying the grain, may reduce the grain's germinability.

3. Insect activity at moistures too low to permit much mold growth may damage or destroy the kernel embryos.

4. Prolonged drying and heating of surface grain by radiated heat under the roof of the bin could conceivably reduce germinability, and might also kill some of the fungi within the grain, especially if the mycelium has not sporulated.

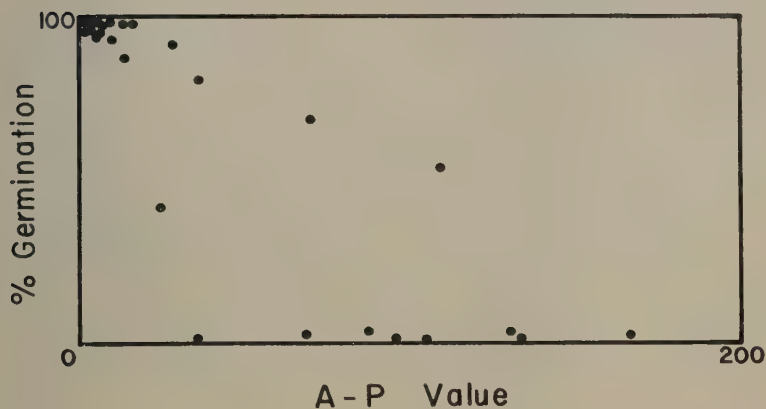


Fig. 3. Deterioration pattern of shelled corn from some CCC bins (stirred grain) located in central Iowa.

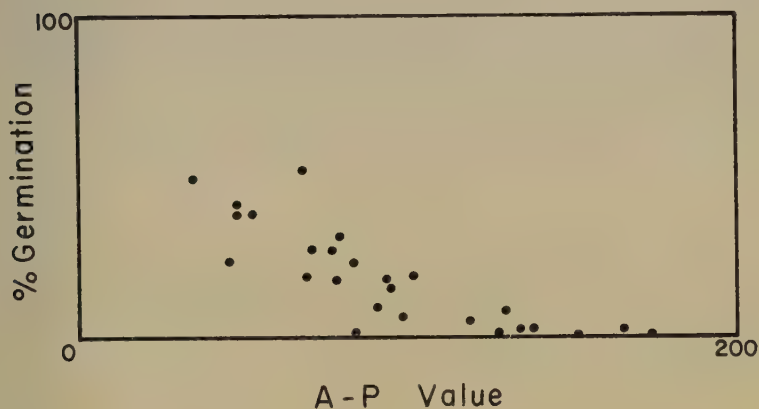


Fig. 4. Deterioration pattern of 1952 shelled corn from some CCC bins (stirred grain) from locations scattered throughout Iowa.

5. The presence of Aspergillus restrictus in kernels, one of the A. glaucus group, often is not detected by our usual methods, because of its very slow growth from inside the grain when identification is made in moist chambers or on nutrient medium. In some samples we do find high counts of this species when the surface-sterilized grain is ground and plated by a technique which we use for quantitative internal mold counts.

6. The presence of certain yeast and bacterial infections within corn may not be easily detected because of their limited growth on the outside of the kernels when the grain is placed in moist chambers or on nutrient medium. Also, these organisms have been observed to inhibit the growth of certain fungi, such as those belonging to the Aspergillus glaucus group; if this is not taken into account it results in a lower A-P value. Candida in particular, a filamentous, non-ascospore-forming yeast, may invade grain which has not dried down sufficiently or rapidly enough, or which has become rewetted during storage, and its visual detection in some situations may require considerable care. However, its presence in significant amounts usually can be detected by a sour or yeasty odor which it imparts to grain kept under moist conditions. Perhaps the presence of Candida is one of the most important factors in cases of very low germination accompanied by relatively low detectable infection by aspergilli and penicillia. A practical method of compensating for the presence of Candida would be to throw the deterioration rating into the next higher category if the grain possesses a sour or yeasty odor.

Figs. 4, 5, and 6 represent three harvest years of corn, 1952, 1953, and 1954, respectively. These three graphs contain data from a total of 540 samples of shelled corn collected during the summer of 1956 from locations scattered throughout Iowa. Each point on the graphs represents the average of the samples from one of thirteen sections of Iowa, and averages for the upper 1 foot of grain and grain from the 4-5 foot level are shown separately.¹ In these graphs we again see, as in Fig. 3, a tendency for rather low germinability accompanied by relatively low A-P values, which contrasts with the pattern for undisturbed grain (Fig. 2).

SUMMARY

A rating scale is proposed for experimental use for expressing quantitatively the degree of deterioration in samples of shelled corn, based on the germinability of the kernels and the amount of infection by species of Aspergillus and Penicillium.

¹ The degrees of deterioration shown in these three graphs and in the other figures in this paper are not intended to represent average deterioration patterns in the respective types of storages or harvest years. The samples were originally chosen with other objectives in mind, and some were biased with respect to the amount of deterioration present.

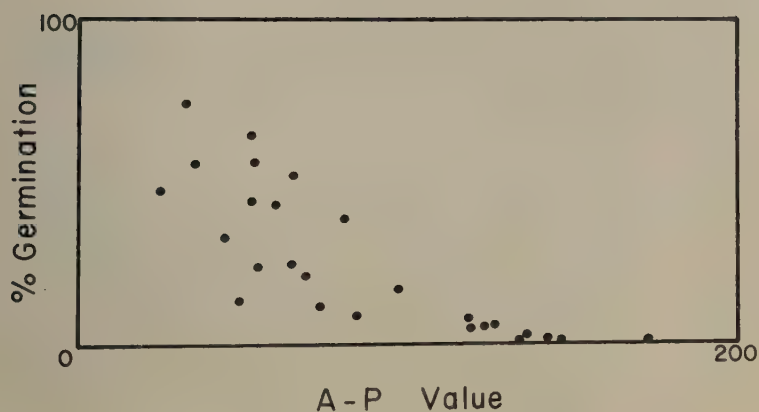


Fig. 5. Deterioration pattern of 1953 shelled corn from some CCC bins (stirred grain) from locations scattered throughout Iowa.

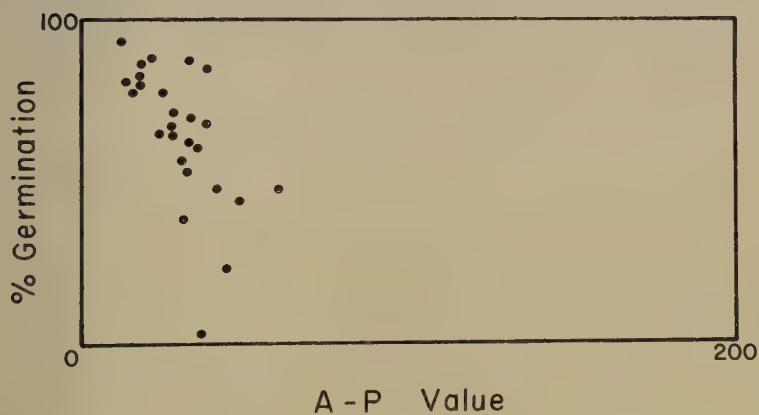


Fig. 6. Deterioration pattern of 1954 shelled corn from some CCC bins (stirred grain) from locations scattered throughout Iowa.

REFERENCE

- Lichtwardt, R.W., G.L. Barron, and L.H. Tiffany. 1958. Mold flora associated with shelled corn in Iowa. Iowa State College Jour. Sci. 33:1-11.

QUANTITATIVE ESTIMATIONS OF THE FUNGI ASSOCIATED
WITH DETERIORATION OF STORED CORN IN IOWA¹

G. L. Barron² and R. W. Lichtwardt³

Quantitative estimations of the molds in cereal grains have been made by several workers but particularly by Christensen and his associates at the University of Minnesota. Christensen (2) developed his technique as a method for counting molds in samples of flour. Briefly, the method consisted of weighing out two grams of flour and suspending this in a known volume of 0.5% saline solution; a dilution series was made from this primary suspension and one millilitre aliquots were pipetted into petri plates, a malt-salt agar added, and the mixture swirled to give an even distribution of the suspension. The results were expressed as molds per original gram of flour (m.p.g.). This method was extended by Christensen and Gordon (4) to estimate the mold flora of stored corn and wheat. In these studies the assays were made by first grinding the cereal to a powder in a Wiley type mill. The mill was cleaned between lots and the first portion ground was discarded to prevent any serious contamination. In later work Bottomley *et al.* (1) using essentially the same technique substituted 0.3% agar for the saline solution as the suspension medium. Quantitative estimations were carried out by Tuite and Christensen (7) on the mold flora of barley but in these studies 2 grams barley were comminuted in a Waring blender in 500 ml of sterile 0.2% agar to give the primary suspension.

In all the above experiments the grain was not surface sterilized prior to grinding and the estimations therefore include fungus material both in and on the grain.

MATERIALS AND METHODS

Experiments were carried out repeating the techniques of earlier workers using a Wiley type mill and the method outlined by Bottomley *et al.* The mill was cleaned thoroughly between samples and swabbed down with 70% alcohol. The sieve was similarly treated and sterilized by flaming lightly. One experiment showed that a sterile sample, following

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² Present address: Department of Botany, Ontario Agricultural College, Guelph, Ontario.

³ Present address: Department of Botany, University of Kansas.

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a highly contaminated sample, picked up over 1000 molds per gram from the mill; moreover, as corn could not be surface sterilized conveniently before grinding this method was discontinued.

Preliminary experiments using surface sterilized corn and a Waring blender showed that this machine could not satisfactorily break down the corn, especially the pericarp and horny endosperm which introduced difficulties in pipetting and dilution work.



Fig. 1. The Sargent centrifugal wet mill No. 2 used for grinding corn samples.

A method was developed using a Sargent centrifugal wet mill No. 2 (Fig. 1) for grinding corn samples. A number of experiments were carried out varying the weight of corn, volume of liquid, and time of grinding. The best results were obtained when 25 gms of corn were ground in 250 ml of 0.2% sterile agar for 35 minutes. From this primary suspension a dilution series was made by pipetting 5 ml aliquots into 45 ml of sterile 0.2% agar in 4 oz medicine bottles and repeating to give a 1/10, 1/100, 1/1000, etc. series. From three selected dilutions, 1 ml aliquots were pipetted in triplicate into petri plates. Malt-salt agar (2% malt extract, 6% salt) was poured into the plates and the mixture swirled to give an even suspension. Plates were incubated three days at room temperature prior to counting and reinspected at intervals to confirm species identifications or observe the appearance of any slow-growing fungi.

The corn was surface sterilized before grinding by immersing for 30 seconds in 70% alcohol followed by two minutes in 1/1000 mercuric chloride and was finally rinsed in sterile distilled water. To prevent external contamination while grinding, a hood was designed and fitted over the grinding parts of the mill. Control samples remained sterile after grinding for one hour and plating out 5 ml replicates of the primary suspension, indicating that the technique gave satisfactory sterile grinding. For most samples external contaminations are not important since, being at a relatively low concentration, they are readily diluted out; however, in comparatively undeteriorated samples they become increasingly important.

After the ground sample is removed, the mill is spun in clean water to remove debris, swabbed down with 70% alcohol, spun for two minutes in 1/1000 mercuric chloride and rinsed in 70% alcohol. The method using the Sargent centrifugal wet mill as outlined above offers two primary advantages over previously used methods: (1) it permits wet surface sterilization of the corn immediately prior to grinding and (2) the mill can be effectively and quickly sterilized between samples.

Samples processed in these studies were collected from Commodity Credit Corporation binsites through the agency of the county A.S.C. offices. Each county agent selected three bins of CCC corn representing one bin each of currently molding 1952 and 1953 corn and one bin of high grade 1954 corn. From each bin, samples were taken representing the upper six inches or top layers of corn and mailed in airtight containers to our laboratory. In all, 256 samples from 95 Iowa counties were collected and processed in the summer of 1956.

RESULTS

The average suitability of corn for storage varies considerably from one year to the next. Thus one year's harvest may show considerably less deterioration than another during the same or even a longer period of storage. Such variations are difficult to express even on a relative basis due to the difficulties in obtaining quantitative data on the molds involved in these different years.

Differences in suitability for storage of 1952 and 1953 harvests are indicated (Table 1) by the greater frequency of the molds significant in deterioration as dominants or co-dominants in the latter years. These differences are much more marked when they are expressed as total mold counts per gram as in Table 2. In every case, with the exception of Aspergillus repens and to some extent A. chevalieri var. intermedius, the counts are much greater from 1953 harvests than from 1952 despite the fact that the latter corn had been stored one year longer. It will be remembered that both these lots of corn were purposely selected as representing moldy corn, and on this basis observed deterioration differences between the two years would tend to be minimized. Thus on an over-all basis, in comparisons between samples collected as being of the same deterioration group, the 1953 corn yielded 4 to 5 times the number of mold counts per gram obtained from the 1952 samples. If counts of Fusarium moniliforme are omitted from totals (on the basis

Table 1. Number of recordings of the principal fungi associated with the deterioration of 256 top samples of stored corn from three harvest years.

Fungus	1952	1953	1954	Number of recordings
* <i>Aspergillus ruber</i>	38	37	12	87
<i>Penicillium</i> spp.	20	27	7	54
<i>Penicillium cyclopium</i>	18	20	15	53
* <i>Aspergillus restrictus</i>	23	25	0	48
<i>Fusarium moniliforme</i>	1	5	30	36
* <i>Aspergillus amstelodami</i>	11	23	5	39
* <i>Aspergillus chevalieri</i>	7	15	6	28
* <i>Aspergillus chevalieri</i> var. <i>intermedius</i>	8	11	6	25
<i>Candida</i> sp.	9	14	2	25
* <i>Aspergillus umbrosus</i>	6	16	0	22
<i>Aspergillus candidus</i>	7	9	5	21
* <i>Aspergillus repens</i>	8	7	3	18
<i>Aspergillus flavus</i>	9	2	2	13
<i>Penicillium viridicatum</i>	4	6	0	10
<i>Aspergillus versicolor</i>	2	5	1	8
<i>Mucor</i> sp.	0	0	6	6
<i>Absidia repens</i>	2	0	2	4
<i>Aspergillus niger</i>	0	1	0	1

* *Aspergillus glaucus* group.

that this fungus is a "field mold") the 1954 samples exhibited 100-400 times less fungus activity than samples from the other two years.

The frequency of occurrence of the different fungi as dominants or co-dominants in Table 1 indicates that both qualitatively and quantitatively the fungus flora responsible for deterioration is approximately the same for the years 1952 and 1953.

The role of the fungi involved in deterioration.

Table 3 gives the total counts and total recordings for the 3 years. Data from all three tables indicate that the species belonging to the *Aspergillus glaucus* group were the most important deteriorators of the stored corn samples tested. They were more numerous than all other species together, both in frequency of occurrence as dominants and co-dominants and on the basis of total mold counts per gram of corn. Frequency of occurrence in Table 1 indicates *A. ruber* was undoubtedly the most ubiquitous species of the group. However, considering total mold counts and average mold counts per occurrence, *A. ruber* does not appear to be as significant in the deterioration process as some of the other species of the *A. glaucus* group, notably *A. restrictus*, *A. chevalieri* var. *intermedius*, and perhaps *A. amstelodami*. This conclusion,

Table 2. Mold counts in 256 samples of stored corn from three harvest years, expressed for each species as the summation of counts in all positive recordings.

Species	Mold counts in thousands			Total Count
	1952	1953	1954	
* <i>A. restrictus</i>	19,912.4	102,167.5	--	122,079.9
<i>A. candidus</i>	11,343.8	81,438.3	13.2	92,795.3
<i>Penicillium</i> spp.	16,409.2	51,457.8	30.9	67,897.9
<i>P. cyclopium</i>	6,482.5	30,698.4	501.4	37,682.3
* <i>A. ruber</i>	6,236.7	29,825.6	90.0	36,152.3
* <i>A. chevalieri</i> var. <i>intermedius</i>	13,289.4	15,902.0	3.8	29,195.2
* <i>A. amstelodami</i>	3,216.5	23,664.4	2.8	26,883.7
* <i>A. chevalieri</i>	195.4	18,546.8	3.4	18,745.6
<i>Candida</i> sp.	1,492.7	8,443.4	11.0	9,947.1
<i>A. flavus</i>	1,503.2	4,962.6	182.7	6,648.5
* <i>A. umbrosus</i>	66.8	3,121.1	--	3,187.9
* <i>A. repens</i>	3,574.6	948.3	8.6	4,531.5
<i>F. moniliforme</i>	32.0	395.1	3,336.2	3,763.3
<i>P. viridicatum</i>	263.6	2,367.9	--	2,631.5
<i>A. versicolor</i>	138.1	2,311.5	1.4	2,451.0
<i>Absidia</i> sp.	511.6	--	20.3	531.9
<i>Mucor</i> sp.	--	--	23.0	23.0
<i>A. niger</i>	--	15.9	--	15.9
Total	84,668.5	376,266.6	**892.5	**461,827.6

** Excluding *F. moniliforme*

* *Aspergillus glaucus* group

however, assumes that variations in sporulation between species of this group reflect differences in vegetative growth and not merely differences in ability to sporulate within the kernel; this relationship will be considered more fully later.

Aspergillus candidus while reported in fewer samples than most of the other primary deteriorators is particularly noteworthy because of its high average mold count per occurrence shown in Table 3. Thus *A. candidus* has a high deterioration potential under suitable conditions. On the basis of low frequency of occurrence as a dominant or co-dominant accompanied by a fairly high average count, *A. flavus* is in the same category as *A. candidus*. *A. flavus*, however, would seem to have the lesser deterioration potential both on the basis of frequency of occurrence and average mold counts.

Penicillium cyclopium proved to be the most important single species of *Penicillium* responsible for deterioration in these studies with *P. viridicatum* being the next most frequent species. A number of other species

Table 3. Total mold counts per gram, number of recordings and average mold counts per occurrence for the important species of molds in 256 top samples of stored corn.

Species	Total counts in thousands	Number of recordings	Average mpg** per recording in thousands
<i>A. candidus</i>	92,795.3	21	4,418.8
* <i>A. restrictus</i>	122,079.9	48	2,543.3
<i>Penicillium</i> spp.	67,879.9	54	1,257.0
* <i>A. chevalieri</i> var. <i>intermedius</i>	29,195.2	25	1,167.8
<i>P. cyclopium</i>	37,673.3	53	710.8
* <i>A. amstelodami</i>	26,883.7	39	689.3
* <i>A. chevalieri</i>	18,745.6	28	669.5
<i>A. flavus</i>	6,648.5	13	511.4
* <i>A. ruber</i>	36,152.3	87	415.5
<i>Candida</i> sp.	9,947.1	25	397.9
<i>A. versicolor</i>	2,451.0	8	306.4
<i>P. viridicatum</i>	2,631.5	10	263.2
* <i>A. repens</i>	4,531.5	18	251.8
* <i>A. umbrosus</i>	3,187.9	22	144.9
<i>Absidia repens</i>	531.9	4	132.9

* *Aspergillus glaucus* group.

** Molds per gram.

of *Penicillium* were reported as being more or less significant in the deterioration process; these have been lumped together under the heading *Penicillium* spp. and include *P. frequentans*, *P. martensii*, *P. variable*, *P. oxalicum*, and *P. palitans*, among occasional others. The high frequency of *P. cyclopium* in all three years suggests a wide latitude of conditions suitable for deterioration by this species and its high average count would indicate a high deterioration potential.

Fusarium moniliforme is fairly low in its occurrence in the highly deteriorated corn of 1953 and 1952. Its rare association with deteriorated corn indicates that it is of little significance in the spoilage of corn in the bins. This species is the most frequent fungus in 1954 corn; apparently conditions in 1954 were favorable for the development of *Fusarium* ear-rot in the field, which accounts for the high frequency and high counts in these samples. Conditions favorable for the development of ear-rot in the field might also account for the high frequency of *P. cyclopium* in 1954 corn.

On a qualitative basis, a number of fungi commonly present in analyses on the same samples by other methods (6) are not present in analyses made using the present techniques. Notable among these are *Diplodia zeae*, *Chaetomium* spp., and *Microascus* spp. to which the medium or the techniques involved are apparently unsuited. Since none of these

fungi were regarded as serious deteriorators of corn, no attempt was made to modify the technique to establish their relative importance on the basis of mold counts.

DISCUSSION

Almost all of the species listed in Table 1 were dominant in one or another of the samples tested. Thus, when conditions are suitable, any of these fungi might have the major responsibility in deterioration of the grain. It is more usual, however, for several fungi to be active as co-dominants in the majority of deterioration complexes. The frequency of a species as a dominant or co-dominant in a wide variety of samples is perhaps a good measure of physiological latitude of the species, particularly with respect to its moisture requirements and CO₂ tolerance. The A. glaucus group, and particularly A. ruber, is outstanding in this regard. Again, average molds per gram per occurrence might be a good criterion for deterioration potential of the different species. On this basis A. candidus and A. restrictus are extremely important, as indicated in Table 3. A. candidus, however, seems more limited in its scope than A. restrictus and the other primary storage molds because of its more exacting physiological requirements as evidenced by its less frequent occurrence as a dominant or co-dominant.

The A. restrictus series is comprised of the slow-growing members of the A. glaucus group. They differ from the other members of the group in that they do not produce cleistothecia but they are included here by Thom and Raper (8) because of certain physiological and morphological similarities to other A. glaucus group species.

Members of this series were first recorded as causing deterioration of grain in storage in 1955 by Tuite and Christensen (7). Working with stored barley, these workers found that at moisture contents from 13.8 to 14.2% there was a slow, gradual increase in the presence of seeds yielding a slow-growing subspecies of the A. glaucus group which they placed tentatively in the A. restrictus series. In a later paper Christensen (3) noted that A. restrictus also invaded seeds of various wheats stored at moisture contents of 13.5 - 15% for sixteen months. Christensen noted that the presence of A. restrictus was difficult to detect in seeds because the fungus grew poorly or not at all on normal culture media. Christensen suggested using media containing 10 - 20% sodium chloride for detection of this fungus.

Prior to the present work A. restrictus has never been recorded as occurring in stored corn. In the present study it is the third most important species, taking frequency of occurrence as the criterion; on the basis of total mold counts it is easily the highest of the A. glaucus group and would seem to be one of the primary deteriorators of stored corn in Iowa.

A. niger, frequently regarded as a storage mold of some importance, is notable by its absence as a dominant in these studies and only once recorded in measurable quantities. It is possible that A. niger is a poor sporulator under the restricted environment of the corn kernel. Koehler (5) noted that A. niger fails to establish itself in corn with less than

18.3% moisture, and that much higher moistures were required for active growth. Thus it would appear that when conditions are not longer limiting for the growth of A. niger the primary molds would already have become well established. In the CCC bins from which the samples were taken, it is the practice to stir the upper grain layers at intervals to distribute accumulated moisture, a practice which would also tend to create conditions unfavorable to the development of A. niger. Stirring may also account for the low frequency of occurrence of A. flavus which is also regarded as a high moisture mold.

The major difficulty in the interpretation of these data, and all quantitative data of this nature, is that it is not at present possible to establish the true relationship between sporulation and growth. It may be largely sporulation which is being measured by these counts, but it is the growth accompanying the sporulation which is responsible for the observed deterioration. While sporulation must be a function of growth, this relationship is bound to fluctuate between species according to habit and within species according to strain differences and environmental effects. All the storage molds have been found to fruit more or less actively within the kernel, particularly in the embryo region, but also to some extent in endosperm cavities. Thus, on the basis of casual observation, there seems little difference in the fruiting abilities of A. candidus, A. restrictus, and P. cyclopius on the one hand and A. flavus, P. viridicatum, and A. umbrinus on the other hand. This would indicate that the observed differences between these two groups of species on the basis of frequency of occurrence and total mold counts may be more related to their true differences as deteriorators than they are to their relative ability to sporulate. Experiments are in progress to establish the relative fruiting abilities of the different species within the kernels.

SUMMARY

1. A technique is outlined for the quantitative estimation of mold flora associated with the deterioration of stored corn in Iowa.
2. Two hundred and fifty-six samples of corn, representing three harvest years from 95 counties in Iowa, were processed by this technique in the summer of 1956.
3. Collections of 1953 corn rated as moldy had significantly higher mold counts than 1952 corn with the same rating, despite the fact that the latter had been stored one year longer.
4. The composition of the fungus flora responsible for deterioration is approximately the same for the two years.
5. The Aspergillus glaucus group appears to contain the most important species of deteriorating stored corn in Iowa, both on the basis of frequency of occurrence as dominants and co-dominants, and on total counts. A. ruber was the most ubiquitous species of the group but A. restrictus, A. chevalieri var. intermedius and perhaps A. amstelodami were probably more important in deterioration.

6. Approximately 25% of the counts were due to species of Aspergillus other than A. glaucus of which A. candidus was the most important single species.
7. Approximately 25% of the total counts were due to Penicillium spp. of which P. cyclopium was the most important single species.
8. Excluding species of the genera Aspergillus and Penicillium only Candida sp. appeared responsible for any serious deterioration.

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THE EFFECT OF SOME ORGANIC COMPOUNDS ON THE
RATE OF THE $\text{Fe(II)} - \text{Fe(III)}$ EXCHANGE¹

Frederick R. Duke and Edward Wolf

Institute for Atomic Research and Department of Chemistry
Iowa State University
of Science and Technology
Ames, Iowa

INTRODUCTION

The exchange reaction between Fe(II) and Fe(III) in perchloric acid solutions has been the subject of several investigations (1, 2, 3). This reaction may be studied by using radioactive tracer iron and following the radioactive exchange: ${}^{\text{Fe}}\text{Fe}^{+++} + \text{Fe}^{++} \rightarrow {}^{\text{Fe}}\text{Fe}^{++} + \text{Fe}^{+++}$. Silverman and Dodson (1) found the reaction to be second order over-all and first order with respect to the total concentration of each oxidation state. The over-all rate constant of the reaction in perchloric acid solutions was found to be a linear function of the reciprocal of the acid concentration.

Much work has been done on the effect of various anions on electron exchange reactions in addition to the ferrous-ferric reactions. Duke and Pinkerton (4) found the expression for the rate of exchange between ferric iron and stannous tin to be fourth order in total chloride concentration. Much of this work was done using simple anions such as the halide ions and pseudo-halide ions (e.g. CN^- , etc.).

The present work presents a study of the effects of some potential organic catalysts on the rate of exchange between ferrous and ferric iron in 1f perchloric acid.

EXPERIMENTAL

Materials:

Perchloric acid (70% vacuum distilled) purchased from J. T. Baker Chemical Company was standardized by titration with standard sodium hydroxide. It gave negative tests for chloride.

Ferric perchlorate obtained from G.F. Smith Chemical Company was used directly to prepare a stock solution of 0.069f ferric perchlorate in 1.50f perchloric acid.

A ferrous perchlorate stock solution was prepared by dissolving electrolytic iron in dilute perchloric acid. The resulting solution was

¹ Contribution No. 781. Work was performed in the Ames Laboratory of the U.S. Atomic Energy Commission.

approximately 0.2f ferrous perchlorate in 2f perchloric acid and generally less than $10^{-3}f$ in ferric perchlorate as determined by titration with sulfatoceric acid. The acid concentration of the ferrous perchlorate stock solution was determined by passing an aliquot of the solution through a cation exchange resin column and subsequently titrating with standard sodium hydroxide. This gave the total perchlorate concentration. Another aliquot was passed through a Jones reductor and titrated immediately with sulfatoceric acid to determine the total iron concentration. Finally, another aliquot was titrated directly with sulfatoceric acid to determine the ferrous iron concentration. The perchloric acid concentration was calculated from the titer containing these three quantities.

Radioactive iron⁵⁹ was obtained from the Oak Ridge National Laboratory. Periodically aliquots of this sample were combined with a predetermined amount of the ferric perchlorate stock solution to give a desired concentration for a particular series of runs.

Procedure:

The reaction was run in a 250 ml Erlenmeyer flask. The flask containing the radioactive ferric fraction was partially immersed in a constant temperature bath. The ferrous fraction was prepared in a separate 125 ml Erlenmeyer flask. The concentrations of the two fractions were nearly the same with respect to all components in any particular run. This was done to minimize any possible effects of mixing. When an organic compound was used it was incorporated into the ferrous fraction.

The reaction was started by rapidly pouring the ferrous fraction into the reaction flask after both fractions had been brought to a constant temperature ($\pm 0.2^\circ\text{C}$) in the bath. The contents of the reaction flask were stirred by a motor-driven glass propeller. At measured intervals an aliquot of 20 ml was withdrawn from the reaction vessel with a 20 ml pipette affixed to a 30 ml hypodermic syringe. With this arrangement samples could be withdrawn and discharged quite rapidly. The aliquot was immediately discharged into a 100 ml beaker which contained 10 ml of dipyridine solution and 10 ml of saturated sodium acetate solution. These amounts were previously determined so as to result in a solution of about pH 5 after the aliquot from the reaction vessel was added. The function of the dipyridine solution was to render the ferrous fraction inert to exchange processes by the formation, rapid at this pH, of the intensely red and highly stable ferrous tris 2, 2' dipyridyl ion.

The ferric iron was then precipitated as iron(III) hydroxyquinolate by adding 10 ml of 8-hydroxyquinoline solution. Because of the low reactant concentrations, an aliquot of inactive ferric perchlorate solution was added following precipitation to increase the mass of the precipitate. The precipitate was filtered, washed, vacuum dried, weighed, mounted on cards, and covered with cellophane. The samples were counted using an end window Geiger-Müller tube connected to a Berkeley 100 Decimal Scaler.

During each run eight aliquots were analyzed. The first six usually were withdrawn within three half-lives at measured intervals of between one hundred and three hundred seconds. The last two were "infinite" time samples which were withdrawn after at least ten half-lives had elapsed; these two samples generally agreed within 4%.

RESULTS AND DISCUSSION

During the preliminary stages of this work, the over-all order of the exchange process was found to agree with that obtained by Silverman and Dodson (1). The time dependence of the fraction of activity exchanged was found to follow the expression:

$$\ln \frac{\bar{F}e_o^{+++} - \bar{F}e_\infty^{+++}}{\bar{F}e_o^{+++} - \bar{F}e_\infty^{+++}} = -R \frac{[Fe^{+++}] + [Fe^{++}]}{[Fe^{+++}] [Fe^{++}]} t,$$

where the terms $\bar{F}e_o^{+++}$, $\bar{F}e^{+++}$, and $\bar{F}e_\infty^{+++}$ represent the specific activity of the ferric fraction at time equal to zero, time t , and "infinite" time, respectively. If F represents the quantity in the logarithmic term, a plot of $\ln F$ versus time gives a straight line whose slope is $[Fe^{+++}] + [Fe^{++}]$

$-R \frac{[Fe^{+++}]}{[Fe^{+++}] [Fe^{++}]}$. Such a graph is given in Fig. 1. The intercept

is zero after correction for zero time exchange. The zero time exchange represented by $(1-F_o) 100$ averaged nearly 60%. If this exchange is reproducible it has no effect on the slope of the lines and only causes variation of the intercept.

When organic compounds such as benzoquinone, pyrazine, and p-phthalic acid were introduced into the system it was thought that catalysis of the reaction might be observed. Catalysis would be the result of the formation of a bridge-type structure which would enhance the transference of an electron. However, it was found that benzoquinone at concentrations comparable to those of the reactants caused no increased rate of exchange. Pyrazine at concentrations comparable to those of the reactants and as high as 0.25f did not affect the rate of exchange.

The introduction of p-phthalic acid into the system caused a marked increase in the exchange rate as shown in Fig. 1. Because of the very low solubility of p-phthalic acid in 1f perchloric acid, the concentration of the p-phthalic acid was not varied. Table 1 summarizes the results.

Taube (5) used the phthalic acids as electron mediators in the reaction of $(NH_3)_5Co^{III}L$ and Cr^{++} where L represents the anion of the acid in solution. Taube found that the para isomer increased the rate considerably while there was a relatively large decrease in catalytic effect when m-phthalic acid was used. The effect caused by o-phthalic acid was very small, probably due to structural hindrances. Analogously, the ferrous-ferric reaction should show the greatest increased rate of exchange with p-phthalic acid.

One may conclude from the present work that p-benzoquinone and pyrazine fail to form complexes with either Fe(II) or Fe(III) or both, but that p-phthalic acid does form a bridged complex. No runs were made with the meta or ortho isomers of phthalic acid. However, one would predict the same decreased catalysis when the para, meta, and ortho isomers are used, respectively, in the ferrous-ferric iron exchange reaction.

Table I

Compound L	Conc. of L. $f \times 10^4$	Conc. of Fe ⁺⁺⁺ $f \times 10^4$	Conc. of Fe ⁺⁺ $f \times 10^4$	Conc. of HClO ₄ f	Temp. °C	Catalysis
Benzoquinone	2.5	2.7	2.7	0.51	+0.2	none
Pyrazine	5.0	5.0	5.0	1.00	+0.2	none
	2.5×10^3	2.5	4.7	1.00	+0.2	none
p-Phthalic Acid	<10	2.5	4.7	1.00	+0.2	40%

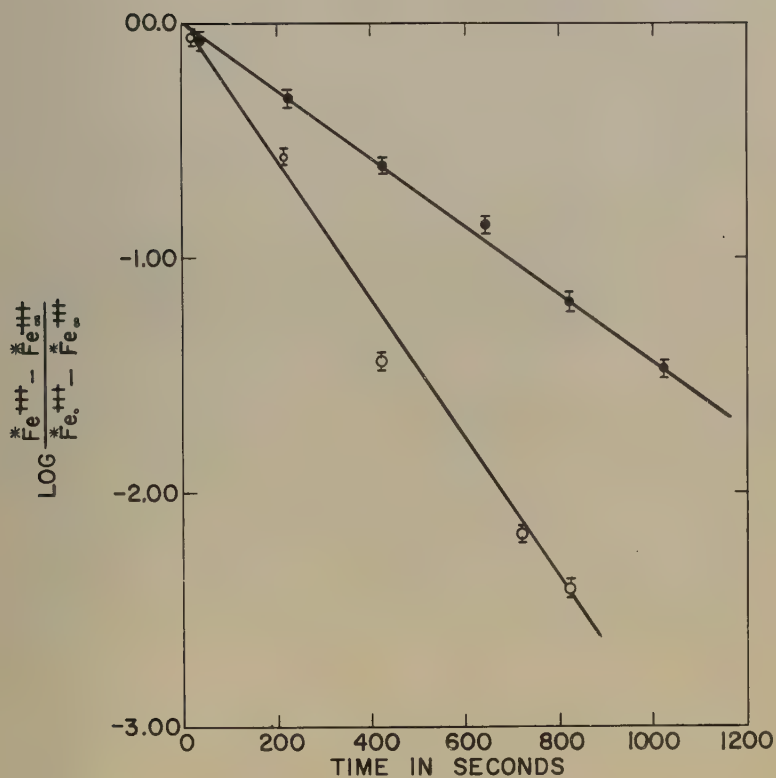


Fig. 1.

● no organic catalyst

○ p-phthalic acid

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SOME REACTIONS OF GELSEMINE

Ernest Wenkert and John H. Hansen

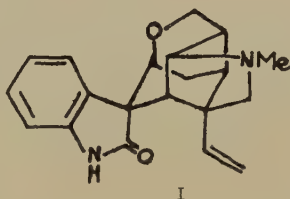
Department of Chemistry
Iowa State University
of Science and Technology
Ames, Iowa

Abstract

Various reactions mainly of the vinyl and oxindole functions of the alkaloid gelsemine are described. Two new methods for opening the oxindole ring are portrayed. The biosynthesis of the alkaloid is discussed.

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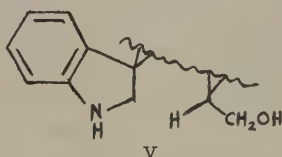
Prior to the recent X-ray determination of the structure of gelsemine (1) and the presentation of chemical and NMR evidence in conformity with this structure (2) experiments were under way in this laboratory designed to penetrate the then mysterious hexacyclic nucleus of this major alkaloid of *Gelsemium sempervirens*, Ait. Whereas many routes of degradation failed, mainly because of the amazing lack of crystallinity of many derivatives, some reactions shed some light on the functional groups of the alkaloid while others opened degradative pathways possibly useful in structure determinations of other oxindole alkaloids. The following discussion will be based on the now established structure I (1, 2) for gelsemine.



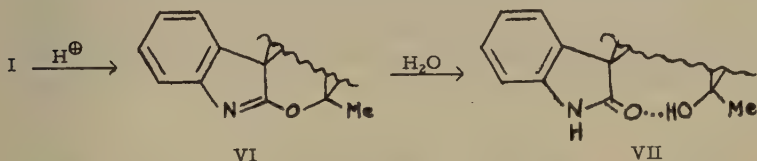
The Vinyl Group.

Early hydrogenation studies (3) revealed the presence of one olefinic linkage in gelsemine. Its location on a terminal methylene group was established by the absence of a C-methyl group in the alkaloid but its presence in the dihydro derivative (4) and by the formation of formaldehyde in the ozonization (5) (an ambiguous result in view of the presence of an equally vulnerable N-methyl group) as well as Lemieux oxidation (6) of the alkaloid. More recently the double bond was shown to be a vinyl group (7, 8), attached to a quaternary carbon atom (9).

a process which now has ample analogy (11).



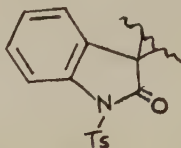
Treatment of gelsemine with hydrochloric, hydrobromic or hydroiodic acids has been reported to yield the hydration products apogelsemine and isoapogelsemine as well as a haloisoapogelsemine (12,13). Each of the halo compounds was convertible into isoapogelsemine (12,13), while zinc reduction of one of them, iodoisoapogelsemine, has yielded dihydrogelsemine (13). These facts are best interpreted on the basis of all products being unrearranged addition compounds. This view was strengthened when, on repetition of a hydrochloric acid treatment of gelsemine, the infrared spectra of the crude reaction mixture and the purified products were inspected. The crude mixture consisted largely of the imino ether, allogelsemine (VI). As a consequence, apogelsemine (VII), the major product, undoubtedly arises from a simple hydrolysis of the imino ether linkage. Both this substance and the epimeric alcohol, isoapogelsemine (VII), exhibited OH absorption only at 3.0μ , characteristic of hydrogen-bonded hydroxyl groups. These data were further corroboration of the propinquity of the hydroxyl and oxindole carbonyl groups.



When, in an attempt to obtain hydrated products without an accompanying halo compound, gelsemine was treated with refluxing 20% sulfuric acid for 19 hours, a new apo compound A was isolated. It was converted into B by sublimation or by attempted Oppenauer oxidation. Compound B could be obtained directly from gelsemine by a similar sulfuric acid treatment for 65 hours. While neither A nor B were identical with apogelsemine or isoapogelsemine, they were presumably isomers of the latter. This could be assured of B by analysis, but only conjectured about A, a compound which along with its derivative resisted all efforts of purification. Undoubtedly, these substances were Wagner-Meerwein rearrangement products. Compound A possessed a nonhydrogen-bonded hydroxyl group and formed an O-acetate. However, B showed strong hydrogen bonding between the hydroxyl and oxindole carbonyl groups and was converted readily to an O, N_a -diacetate. Formation of the latter is in sharp contrast to the strong resistance of gelsemine toward N_a -acetylation (5). Various attempts of oxidation of compounds A and B led to indefinite results.

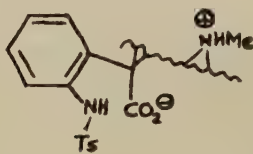
The Oxindole Moiety.

Another entry into the center of the polynuclear structure of gelsemine appeared to be via the oxindole unit. If the latter could be ruptured, in principle a path might be found for complete removal of this moiety. Since the oxindole lactam had been found to be exceedingly resistant to hydrolysis (13), it had to be altered in a manner that would make the carbonyl group more vulnerable to nucleophilic attack and make the resulting o-aminophenylacetic acid system more stable. Both these features could be predicted for and, indeed, were found in N_a -tosylgelsemine (VIII). The latter was formed in a manner analogous to the reported synthesis of N_a -methylgelsemine (14). The crystalline derivative exhibited its carbonyl peak in the infrared spectrum at an expected lower wavelength, 5.70μ . It readily formed a methiodide, which on attempted Hofmann degradation under various conditions yielded no characterizable products.

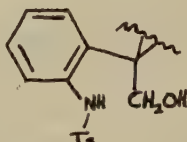


VIII

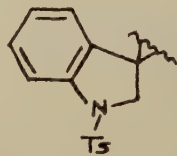
Whereas tosylgelsemine (VIII) was stable toward dilute acid, it was converted easily by base into an amino acid (IX). The latter reverted to tosylgelsemine (VIII) on treatment with 50% sulfuric acid at room temperature. Lithium aluminum hydride reduction of VIII yielded the amino alcohol (X), rather than N_a -tosyldihydrodesoxogelsemine (XI), a substance which was prepared by tosylation of dihydrodesoxogelsemine. Thus, two different schemes for opening the oxindole moiety of the alkaloid had been found.



IX



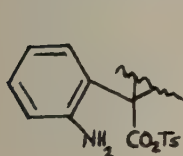
X



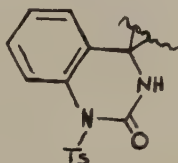
XI

When the amino acid (IX) was refluxed in an ethylene glycol solution of sodium hydroxide, it reverted to gelsemine (I). The ease of this dehydrative detosylation can be accounted for by assuming a prior intramolecular N-to-O transtosylation and internal amination of the resulting mixed anhydride (XII). Exposure of IX to concentrated sulfuric acid and hydrazoic acid, in an attempted Schmidt reaction, led to a noncrystalline mixture of tosylgelsemine (VIII) and presumably the cyclic urea (XIII). Unfortunately, separation of the latter and, hence, its structure proof failed. Treatment of IX with iodine and sodium bicarbonate yielded an amorphous substance, assumed to be iodolactone XIV. Its 5.77μ infrared

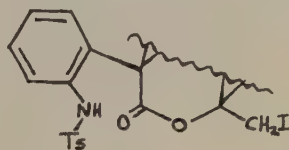
carbonyl peak indicated it to be a δ -lactone, a ring of a size now to be expected on the basis of the accepted structure I for gelsemine.



XII

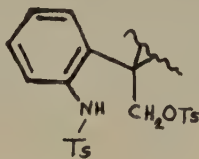


XIII



XIV

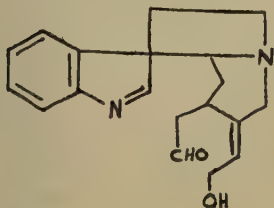
An attempt to convert the α,α -disubstituted β -(*o*-tosylamidophenyl)-ethanol X to its corresponding styrene by treatment with formic acid led to unidentifiable products. Likewise, solvolysis of its tosylate (XV), prepared by lithium aluminum hydride reduction of tosylgelsemine (VIII) and tosylation of the unseparated reaction mixture, met with failure. Similar reactions with products derived from dihydrogelsemine led to no more success.



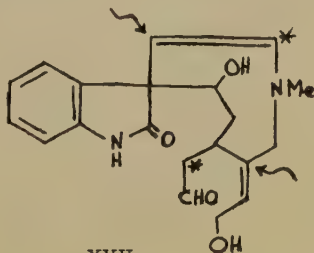
XV

Biosynthesis of Gelsemine.

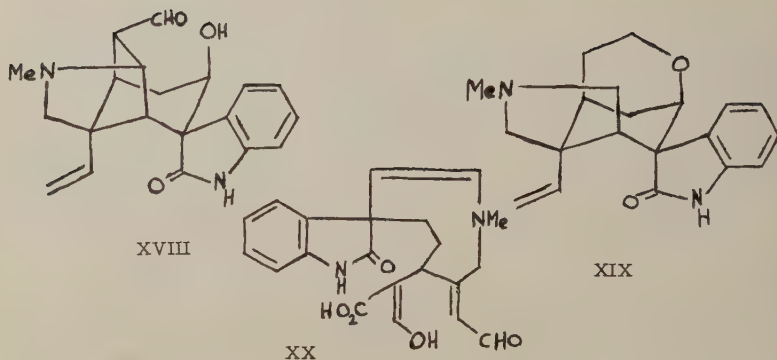
In a brilliant exposition of biosynthetic theory Woodward was able to derive the structure of gelsemine prior to its complete elucidation (1, 15). By analogy with the biosynthesis and some structural patterns of the strychnos alkaloids the biosynthetic intermediate XVI was considered to lead via several, mainly oxidative operations to XVII. An intramolecular carbon-carbon bond formation of a novel type (arrows in XVII) and another of the ajmaline type (asterisks in XVII) were envisaged to yield a hydroxyaldehyde (XVIII), which on reduction and dehydration would give gelsemine (XIX). A slight variant of this scheme, involving XX in place of XVII, has been presented by Conroy (2).



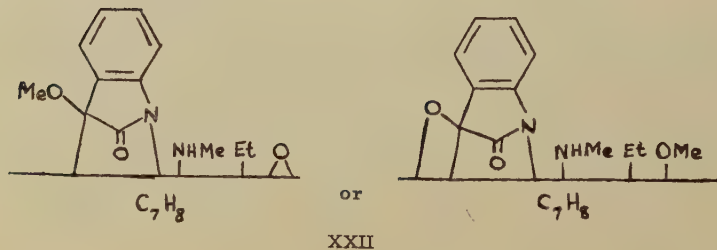
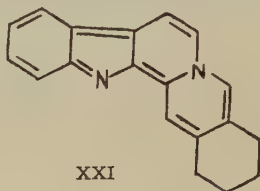
XVI

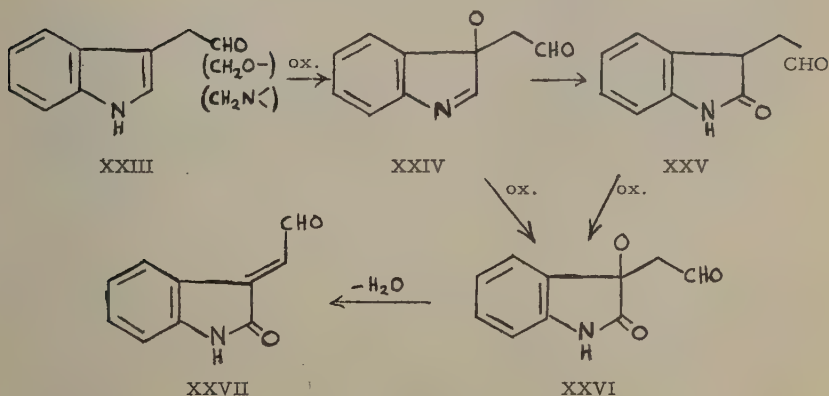


XVII

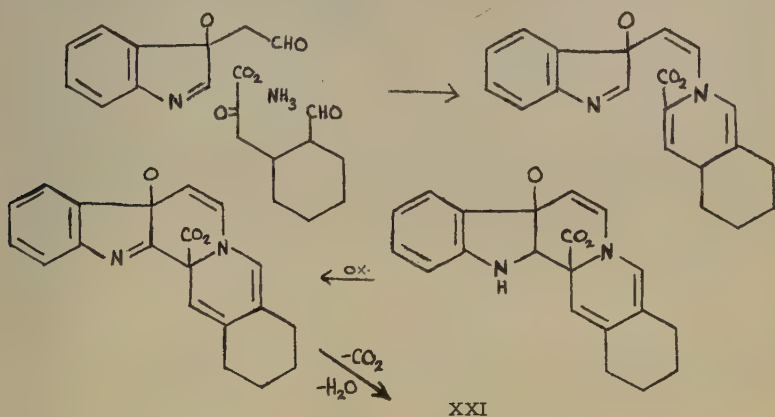


The Woodward scheme is in harmony with the recent generalized theory of alkaloid biosynthesis (16,17), since XVI is readily derivable from tryptophyl, formaldehyde and prephenate units. It places the prephenate moiety in a state of oxidation equivalent to that in the strychnos alkaloids, but higher than in most other indole alkaloids. Inspection of the structures of sempervirine (XXI) and gelsedine (XXII) (18), two alkaloids occurring naturally side by side with gelsemine, however, suggests strongly that the oxidases in the *Gelsemium* plant operate mainly on the tryptophyl residue, rather than the prephenate unit. If, as a consequence, it be assumed that 3-indolylacetaldehyde (XXIII), or its tryptophol or tryptamine counterparts, may proceed through an oxidation series, involving the indolenine XXIV, oxindole XXV, or dioxindole XXVI directly, and oxindole XXVII, biosynthetic intermediates would be on hand, all of which could lead to the various *Gelsemium* and other oxindole alkaloids on condensation with formaldehyde-prephenate systems.

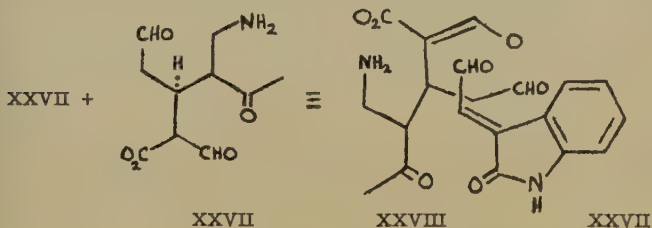


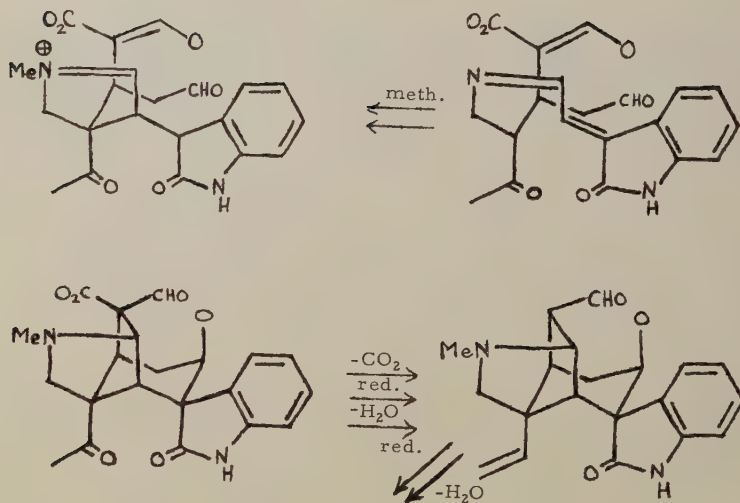


Compounds XXIII and XXIV have the same relationship to each other as cinchonamine and quinamine. Most probably, XXIV serves as precursor of sempervirine:



Intermediates of the type of XXV are the progenitors of rhyncophylline and similar oxindole alkaloids, while XXVI may lead to gelsedine (XXII). Finally XXVII in combination with XXVIII yields gelsemine:





XIX

The above as well as Woodward's suggestion requires the absolute configuration of gelsemine to be as depicted in XIX rather than I.

Acknowledgements. The authors are most grateful to the Upjohn Co. for financial support and for the extraction and isolation of gelsemine, to S.B. Penick and Co. for a sample of the alkaloid and to the Institute of Atomic Research for the use of a Baird infrared spectrophotometer.

Experimental

(Melting points are uncorrected. Ultraviolet spectra were run in 95% alcohol solutions. Optical rotations were measured at 25° in pyridine solutions.)

The Diacetate of Compound V.—A solution of 560 mg of V (Calcd. for 1 C-Me: 4.66; found: 0.54, 0.0) in 10 ml of acetic anhydride and 10 ml of pyridine was heated on a steam bath for 3 hours and allowed to stand at room temperature for 18 hours. After vacuum removal of the solvent the residue was partitioned between dilute sodium bicarbonate solution and chloroform. The organic layer was dried over magnesium sulfate, filtered and evaporated. The residue was chromatographed on neutral alumina and a major fraction, 510 mg, was eluted with 3:1 benzene-chloroform. Whereas this fraction could not be crystallized, vacuum evaporation of its chloroform solution produced a white foam, m.p. 100-105°; infrared spectrum (CHCl₃), C = O 5.80 (s), 6.07 (s) μ , C = C 6.28 (m) μ .

The N-Acetyl Derivative of Compound V.—A solution of 510 mg of the diacetate of V and 53 mg of sodium hydroxide in 4 ml of water and 25 ml of methanol was allowed to stand at room temperature for 21 hours. After vacuum removal of the solvents the residue was dissolved in chloroform and dilute sodium carbonate. The separated organic solution was dried, filtered and evaporated. The residue was chromatographed on alumina. Elution with 1:1 benzene-chloroform yielded a major fraction which could not be induced to crystallize. Drying under vacuum gave a foam, m.p. 115-120°, $[\alpha]_D -33.6^\circ$; infrared spectrum (CHCl_3), OH 2.80 (w) μ , C = O 6.08 (s) μ , C = C 6.29 (m) μ .

Hydration of Gelsemine, Compound A.—A solution of 1.18 g of gelsemine, 10 ml of conc. sulfuric acid and 10 ml of water was refluxed for 19 hours. The solution was cooled, made basic with dilute sodium hydroxide and extracted in 10 portions with 1.05 liter of chloroform. The combined organic extracts were dried over magnesium sulfate, filtered and evaporated to dryness. Attempted crystallization of the residue with acetone produced 520 mg of a white powder, $[\alpha]_D 0.2^\circ$; infrared spectrum (nujol). OH 2.8 (m) μ , NH 3.07 (m), 3.20 (m) μ , C = O 5.89 (s) μ , C = C 6.19 (m), 6.29 (m) μ .

A solution of 150 mg of compound A, a few drops of pyridine and 1 ml of acetic anhydride was heated on the steam bath for 3 hours. After letting the solution stand in the presence of water, it was made basic with dilute sodium carbonate solution and extracted with four 40 ml portions of chloroform. The combined organic extracts were dried, filtered and evaporated. The residue was chromatographed on neutral alumina, the major fraction being eluted with 1:1 benzene-chloroform. It could not be crystallized from common solvents, but deposited crystals on slow evaporation from an acetone solution; m.p. 230° dec.; spectra: ultraviolet, λ_{max} 252 μ ($\log \epsilon$ 3.87) and 283 m μ ($\log \epsilon$ 3.21); infrared (CHCl_3), C = O 5.80 (s), 5.86 (s) μ , C = C 6.28 (m) μ .

Compound B.—a.) When gelsemine was exposed to the conditions of hydration, which led to compound A, but for a longer reaction time, 65 hours, compound B could be isolated. Crystallization from chloroform gave an amorphous white powder. b.) Sublimation of compound A at 200° and 1 μ gave B. c.) Compound B also could be obtained by refluxing for 23 hours a solution of 720 mg of gelsemine and 7.2 g of cyclohexanone in 50 ml of anhydrous benzene, to which a solution of 4 g of aluminum phenoxide in 30 ml of anhydrous benzene had been added, washing the cooled solution with water, extracting the organic products with chloroform and following with the usual work-up.

Two sublimations of B at 220° and 1 μ yielded a powder, m.p. 285-287° dec. (browning at 245°), $[\alpha]_D 4.9^\circ$; infrared spectrum (nujol), OH 2.94 (m) μ , NH 3.08 (m) μ , C = O 5.95 (s) μ , C = C 6.21 (m), 6.31 (w) μ . When compound B was regenerated from its picrate and crystallized in chloroform, it gave small crystals, m.p. 268° dec. (browning at 248°).
Anal. Calcd. for $\text{C}_{20}\text{H}_{24}\text{O}_3\text{N}_2$: C, 70.56; H, 7.11; N, 8.23. Found: C, 69.81; H, 7.44; N, 7.82.

The picrate of B was crystallized three times from ethanol and dried under vacuum at 78° for 4 hours, m.p. 273° dec. (browning at 200°), $[\alpha]_D 34.8^\circ$.

Anal. Calcd. for $C_{26}H_{27}O_{10}N_5 \cdot C_2H_6O$: C, 54.63; H, 5.40; N, 11.38. Found: C, 55.01; H, 5.81; N, 11.27.

A solution of 100 mg of compound B and 2 ml of pyridine in 2 ml of acetic anhydride was heated on the steam bath for 2 hours. The reaction mixture was cooled, diluted with 30 ml portions of ether, the combined extracts dried over magnesium sulfate, filtered and evaporated and the residue crystallized twice from ether-petroleum ether. Further crystallization from ether gave white crystals, m.p. 196-198°, $[\alpha]_D -7.7^\circ$; spectra: ultraviolet, λ_{max} 230 m μ ($\log \epsilon$ 4.03), $\lambda_{shoulder}$ 260 m μ ($\log \epsilon$ 3.48), 277 m μ ($\log \epsilon$ 3.04) and 291 m μ ($\log \epsilon$ 2.62); infrared (nujol), C = O 5.72 (s), 5.78 (s), 5.89 (s) μ , C = C 6.24 (m) μ .

Anal. Calcd. for $C_{24}H_{28}O_5N_2$: C, 67.90; H, 6.65; N, 6.60. Found: C, 68.01; H, 6.49; N, 6.54.

N_a -Tosylgelsemine (VIII).—A mixture of 3.08 g of gelsemine and 0.40 g of potassium in 100 ml of anhydrous benzene was stirred vigorously and refluxed under nitrogen for 16 hours. After addition of 1.84 g of p-toluenesulfonyl chloride in 40 ml of anhydrous benzene to the cream-colored suspension the stirring and refluxing was continued for 8 hours. A solution of 50 ml of water and 35 ml of methanol was added slowly, the mixture stirred for an hour at room temperature, the aqueous layer brought to pH 10 by the addition of sodium hydroxide and the layers separated. The organic solution was washed with water and the aqueous layer with two 50 ml portions of benzene. The combined organic extracts were dried over magnesium sulfate, filtered and chromatographed on neutral alumina. The major product was eluted with benzene, while a small amount of unreacted gelsemine was obtained from the chloroform eluates. Four crystallizations from ether gave 2.35 g (52%) of white needles, m.p. 133-135°, $[\alpha]_D -28.4^\circ$; spectra: ultraviolet, $\lambda_{shoulder}$ 230 m μ ($\log \epsilon$ 4.19), 289 m μ ($\log \epsilon$ 2.85), infrared ($CHCl_3$), C = O 5.70 (s) μ , C = C 6.12 (w), 6.25 (m) μ . Concentration of the mother liquors gave an additional 1.40 g (31%) of crude product, m.p. 130-134°.

Anal. Calcd. for $C_{27}H_{28}O_4N_2S$: C, 68.05; H, 5.92; N, 5.88. Found: C, 67.98; H, 5.95; N, 6.05.

A chloroform solution of N_a -tosylgelsemine (VIII) and excess methyl iodide was refluxed for 1 hour. Removal of the solvent left a residue, which on three crystallizations from methanol yielded white flakes of crystalline methiodide, m.p. 250-252° dec., $[\alpha]_D 20^\circ$.

Anal. Calcd. for $C_{28}H_{31}O_4N_2SI$: C, 54.37; H, 5.05; N, 4.53. Found: C, 54.43; H, 5.37; N, 4.47.

Amino Acid IX.—A solution of 162 mg of sodium hydroxide in water was added to 500 mg of N_a -tosylgelsemine (VIII) in 40 ml of ethanol and the mixture refluxed for 22 hours. After partial concentration of the mixture it was acidified with hydrochloric acid and sodium bicarbonate added. The cloudy solution (whenever a precipitate formed, it was filtered and worked up in the following manner) was extracted with ten 60 ml portions of chloroform and the resulting solution dried over sodium sulfate. After filtration and solvent removal 450 mg (87%) of white solid was obtained, which on four crystallizations from methanol gave 250 mg of white crystals, m.p. 185-187°; spectra: ultraviolet, $\lambda_{shoulder}$ 223 m μ ($\log \epsilon$ 4.22) and 280 m μ ($\log \epsilon$ 3.26), infrared ($CHCl_3$), OH, NH (3.22(w) μ , \oplus NH broad 4.06-4.35 (m) μ , C = O 6.22 (s) μ , C = C 6.30 (m) μ . The analytical sample was dried under vacuum at 100° for 3 hours.

Anal. Calcd. for $C_{27}H_{30}O_5N_2S \cdot CH_3OH$: C, 63.86; H, 6.51; N, 5.32. Found: C, 63.93; H, 6.45; N, 5.50.

Concentrated sulfuric acid, 5 ml, was added to a suspension of 100 mg of the amino acid IX in 5 ml of water and the mixture allowed to stand at room temperature for 4 hours. The mixture was poured onto 15 g of ice and the slurry extracted with 175 ml of chloroform in four portions. The extracts were washed with 5% sodium carbonate solution, dried over magnesium sulfate and evaporated under vacuum. A 1:1 petroleum ether-benzene solution of the residue was chromatographed on neutral alumina, which led to a benzene eluate of 50 mg whose infrared spectrum was identical in all respects with that of N_α -tosylgelsemine (VIII).

A solution of 9.5 mg of sodium hydroxide in 2.5 ml of water was added to a solution of 50 mg of the amino acid IX in 50 ml of ethylene glycol. The mixture was distilled up to a vapor temperature of 193° and then refluxed under nitrogen for 2 hours. Thereupon the solvent was removed under vacuum, the residue dissolved in dilute hydrochloric acid (pH2) and the solution extracted with chloroform. After addition of sodium bicarbonate the aqueous solution was extracted further with four 50 ml portions of chloroform. The combined organic extracts from the neutral solution were dried over sodium sulfate, filtered and evaporated. The remaining white solid, 30 mg, had an infrared spectrum identical with that of gelsemine. Crystallization from acetone gave white crystals, m.p. $173-177^\circ$, m.m.p. $174-177^\circ$ with gelsemine acetonide.

Iodolactone XIV. — A mixture of 50 mg of amino acid IX, 26 mg of iodine, and 1 g of sodium bicarbonate in 50 ml of chloroform was allowed to stand in the dark for 16 days. The colored mixture was filtered, evaporated under vacuum and the residue chromatographed on neutral alumina. Elution with 1:1 benzene-chloroform yielded an oily brown solid and an amorphous white solid whose infrared spectrum ($CHCl_3$) exhibited peaks at 2.8(w), 2.97(w), 5.77(s), and 6.27(m) μ .

Amino Alcohol X. — A mixture of 605 mg of N_α -tosylgelsemine and 45 mg of lithium aluminum hydride in 50 ml of anhydrous dioxane was refluxed for 16 hours under nitrogen. After hydrolysis with 10 ml of 90% methanol, the reaction mixture was evaporated to dryness under vacuum. The residue was dissolved in 40 ml of 5% hydrochloric acid and the solution stirred with 40 ml of chloroform while it slowly was made basic with sodium carbonate. The resulting slurry was filtered and the solid washed twice with 40 ml portions of boiling chloroform. The two phases of the combined filtrates were separated and the organic phase dried over sodium sulfate, filtered and evaporated under vacuum. The residue was crystallized six times from benzene and dried under high vacuum at 100° for 4 hours; m.p. $265-266^\circ$ dec., $[\alpha]_D -88.3^\circ$; spectra: ultraviolet, λ_{max} 221m μ ($\log \epsilon$ 4.20) and 255m μ ($\log \epsilon$ 3.75), λ_{min} 248m μ ($\log \epsilon$ 3.74); infrared ($CHCl_3$), OH 2.80(w) μ , NH 3.05(w) μ . C = C 6.16(w), 6.26(m) μ .

Anal. Calcd. for $C_{27}H_{32}O_4N_2S$: C, 67.48; H, 6.71; N, 5.83. Found: C, 68.01; H, 6.38; N, 5.83.

N_α -Tosyldihydrodesoxogelsemine (XI). — A solution of 170 mg of dihydrodesoxogelsemine, m.p. $138-139^\circ$ [reported (19) m.p. $137.0-137.5^\circ$], and 140 mg of tosyl chloride in 10 ml of pyridine was warmed on the steam bath for half an hour and then left standing at room temperature

for 12 hours. After the addition of 10 ml of water the mixture was concentrated and the resulting pink syrup partitioned between chloroform and dilute sodium hydroxide solution. The organic extract was dried over magnesium sulfate, filtered and evaporated to dryness. Chromatography of the residue on neutral alumina led to a solid product in the 4:1 benzene-chloroform eluates. Three crystallizations from ether yielded white needles, m.p. 153-155°, $[\alpha]_D^{25} -44$; ultraviolet spectrum, $\lambda_{\max} 221m\mu$ ($\log \epsilon$ 4.18) and $262m\mu$ ($\log \epsilon$ 3.84), $\lambda_{\min} 245m\mu$ ($\log \epsilon$ 3.75).
 Anal. Calcd. for $C_{27}H_{30}O_3N_2S$: C, 70.10; H, 6.54; N, 6.06. Found: C, 70.39; H, 6.52; N, 6.10.

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¹ A circulating copy of each complete thesis is available in the Iowa State University Library, Ames, Iowa. Abstracts of these theses are published in Dissertation Abstracts. A microfilm or a photostat copy of a thesis may be purchased from Dissertation Microfilms, 313 N. First Avenue, Ann Arbor, Michigan.

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PUBLICATIONS OF MEMBERS OF THE STAFF
OF THE IOWA STATE UNIVERSITY FOR
THE ACADEMIC YEAR 1958-59

Certain summaries and indices are of interest in a survey of the publications of members of the staff of an educational and research institution such as the Iowa State University. The publications are listed in alphabetic order under the names of the senior authors. Junior authors are also listed alphabetically with cross reference to senior author.

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Number of individuals listed	757
Number of publications	813
Number of publications with single author.	351
Number of publications with joint authorship	462
Number of departments or fields represented in publications	51
Number of individuals who serve as editors or on the editorial staff of one or more scientific or technical periodicals	47

Individuals thus serving are: Atkins, Ayres, Barnes, Bear, Biester, Bird, Black, C. A., Buchanan, R. E., Burchinal, Carlander, Dahm, Davis, Diehl, Dwelle, Fassel, Getty, Gilman, H., Handy, Heath, Heer, Hughes, Hurley, Johnson, I. J., Kempthorne, Kenkel, Kirkham, Knight, Koupal, Kuetemeyer, Kutish, Larson, W. E., Lockhart, Loomis, Mahlstede, J. D. McNabb, Phillips, Rothenbuhler, Simons, Smith, F. G., Snedecor, Swanson, Swenson, M. J., Thielman, Tintner, Wardle, Weber, Werkman.

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